the onset of the desiccation. Then the mRNAs level remained low from stage 9.1 through 9.5. The dehydrin mRNA levels rose again late in development, from stage 9.6 on, apparently dropping in very late development. A similar pattern of expression was observed in a parallel experiment when our lea-like clone, LPZ-216, was used as a probe.

[0156] This pattern reveals two significant peaks at the early development of the embryos and high expression levels for the stage 9.6 and beyond. The expression pattern of these two lea genes in loblolly pine embryos is consistent with the changes in ABA concentration observed in pine during embryogenesis. (See Figure 5)

EXAMPLE 4: Evaluation of Metabolic State of Somatic Embryos as Compared to Zygotic Embryos for Fitness Determination

[0157] The model and goal for somatic embryogenesis is to produce an embryo that in vigor, germinatability, etc., resembles a zygotic embryo. Standard measurements reveal relatively little about the embryos; thus the metabolic state of somatic and zygotic embryos is unknown. The metabolic state of zygotic (natural) embryos can be evaluated by DNA arrays containing the cDNA clones described in this application. A database of mRNA levels for the genes represented on the DNA arrays can then be established. Embryos growing under a new tissue culture protocol (Fig.6, box #2) can be evaluated by DNA array southerns (Fig.6, box #3). The array elucidates patterns of gene activity and reveals whether those patterns are similar to the natural state (Fig.6, box #4). The germination, or further development can be checked (Fig.6, box #5) to confirm the conclusion. Where a link between specific gene activity and embryo performance has been demonstrated, protocols can be modified with efficiency as seen in Figure 6, box 6.

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[0158] To illustrate this process, elevation of plant hormone ABA in maturation medium was evaluated as a protocol modification, as described below. This modification proved beneficial, elevating the number and quality of the embryos produced. The mRNA abundance for cDNAs was assessed by DNA array using RNA isolated from control and elevated ABA conditions; several differences were observed in the mRNA levels of specific genes. Further, abundance of mRNA in the elevated ABA condition, more closely resembled the mRNA abundance observed for the these same genes in zygotic embryos. Thus a protocol which produces higher quality embryos produces, in these embryos, a mRNA profile that more closely resembles that observed in natural embryos.

Zygotic and Somatic Loblolly Pine Embryos

[0159] Loblolly pine cones were collected weekly from a breeding orchard near Lake Charles, Louisiana, and shipped on ice for experimentation. Embryos were excised and evaluated for developmental stage (Pullman et al. 1994). Stage 9 embryos were separated by the week they were collected - 9.1 (week 1), 9.2 (week 2), etc. Staged zygotic embryos were sorted into vials partially immersed in liquid nitrogen and stored at -70°C. Somatic embryos for loblolly pine were initiated as described by Becwar et al. (1995) or with minor modifications. Somatic embryos were grown, selected, and staged as described by Pullman et al. (1994) and stored at -70°C.

Mass Isolation of Genes Differentially Expressed in Loblolly Pine Zygotic Embryos

[0160] The following RNA differential display method is sensitive enough to produce banding patterns from one mid- to late-stage embryo or 10-20 early stage embryos. This technique, which extracts mRNA directly from tissue using oligo(dT)

beads, avoids losses inherent in conventional RNA extraction methods, is fast, reliable, and inexpensive. Differences in gene expression during development, as well as between somatic and zygotic embryos, can be easily detected.

[0161] To achieve these results, 50-100 μl lysis buffer containing 100 mM Tris-HCl, pH 8.0, 500 mM LiCl, 10 mM EDTA, 1% SDS and 5 mM DTT was added to 10-100 mg of staged embryos in a 1.5 ml tube. The mixture was ground thoroughly with an electric drill containing a plastic pestle bit (VWR, Cat# KT95050-99) that had been sterilized by autoclaving. An additional 50-100 μl lysis buffer was added and ground briefly. The grinder and vortex was washed with 100 μl lysis buffer. If multiple samples were processed, each is stored on ice until ready for the next step. The grinding tip was washed with sterile water and dried for the next sample.

[0162] After all the samples were ground, they were spun at 4°C for 15 minutes in a bench top centrifuge at 14,000 rpm. 8 μl oligo(dT) coated Dynal beads (mRNA DIRECT Kit, Dynal, NY) was placed in a 1.5 ml tube. The Dynal beads were washed twice with a 100 μl of the above mentioned lysis buffer and suspended in an equal volume of the lysis buffer used in tissue grinding. If more than one sample is handled, the beads for all the samples can be washed together and dispensed in several 1.5-ml tubes. The cleared embryo lysate (after centrifugation) was added to the beads and mixed well.

[0163] The mixture was then incubated on ice for 5 min., placed on a magnetic stand (Promega) for 5 min., and partially dried by careful removal of the liquid. To this, 100 μl of washing buffer with LiDS containing 100 mM Tris-HCl, pH 8.0, 0.15 mM LiCl, 1.0 mM EDTA, and 0.1% SDS was added. (mRNA DIRECT kit.) The mix was

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